the surface of T cells within 5 minutes of addition of TDB, and the maximal degree of shedding occurs within 2 hours. This is in contrast to CD69, which continues to increase in expression up to 24 hours after TDB addition (FIG. **3**). While both markers show similar sensitivity to TDB (FIG. **2**), the changes in CD62L expression are far more proximal to TDB addition and therefore are a more direct readout of cellular synapse formation.

[0189] The formation of synapse and the linkage to downstream pharmacological effects have been explored in the previous studies (Brischwein, K., B. Schlereth, et al. (2006) "MT110: A novel bispecific single-chain antibody construct with high efficacy in eradicating established tumors." Molecular Immunology 43: 1129-1143; Speiss, C., M. Merchant, et al. (2013) "Bispecific antibodies with natural architecture produced by co-culture of bacteria expressing two distinct half-antibodies." Nature Biotechnology 31: 753-758; and Chen, X., et al. (2016) Mechanistic Projection of First-in-Human Dose for Bispecific Immunomodulatory P-Cadherin LP-DART: An Integrated PK/PD Modeling Approach. Clin Pharmacol Ther. 100(3):232-41). In these studies, formation of synapse was unable to quantitate and was modeled at molecular level with several assumptions: 1) fixed target expression level; 2) fixed CD3 expression level; 3) the calculated total amount of cell-bound target and CD3 are evenly distributed in a well-stirred system as free soluble molecules. The model-predicted molecular synapse was then used to drive the downstream pharmacological effects (e.g. cell killing and T cell dynamics). While this modeling strategy has demonstrated its value in supporting MABEL dose selection and describing the PK-PD relationship in vitro and in vivo, several limitations have been noted.

[0190] First, the relationship between the model-predicted molecular synapse and pharmacological effects might be different depending on the target or CD3 expression level per cell. For example, the total amount of the target could be the same under the conditions of i) low cell density of high target-expressing cell vs. ii) high cell density of low target-expressing cell. At certain concentration of bispecific molecules, the amount of model-predicted molecular synapse will be the same, while the pharmacological effects observed could be different due to different cell density. Second, the models used to predict molecular synapse formation assumed the target and the CD3 as free soluble molecules. However, it is anticipated that the bispecific molecule would have different accessibility to cell-bound molecules compared free soluble ones, and thus the cell density needs to be taken into account. Furthermore, given the pharmacologic effects of T cell dependent bispecific molecule were triggered by T cell activation, the relative cell density between target and effector cells needs to be also taken into account. Third, instead of molecular synapse, it is the formation of cellular synapse structure (i.e. bispecific molecules—target cell—T cell) that triggers the downstream pharmacological activities. While formation of molecular synapse (i.e. bispecific molecule—cell-bound target molecule—cell-bound CD3 molecule) on cell surface is prerequisite, the minimal amount of molecular synapse required for cellular synapse structure remains unclear.

[0191] The objective of current modeling work is to develop a comprehensive model to describe cellular synapse formation, which was approximated by in vitro assay as described above. The datasets generated cover a wide range of factors potentially impacting cellular synapse formation, including 1) target expression level (1,200 copies per cell ~122,000 copies per cell); 2) effector to target cell ratio (1:10~1:0.01); 3) total cell density ($1\sim11\times10^6$/mL). As shown in FIG. **5**, the mechanism-based model developed here used a single uniform model structure to describe multiple interrelated factors and their impact on cellular synapse formation. Through the integrated analysis, the model can provide a framework to assist the discovery and development of T cell dependent bispecific molecule, such as molecule design and candidate selection. The information of the dynamic range of tumor target expression level as well as expression difference between tumor and normal cells can also be incorporated to guide suitability assessment of the tumor target and the rational molecule design of the corresponding T cell dependent bispecific molecule. Through the exercise, the therapeutic windows can hopefully be widened by maximizing the tumor cell killing at the site of action and minimizing unwanted immune response and cytotoxicity to normal cells.

Example 2—Elucidating the MOA of Bispecific Antibodies via a Mechanism-Based Model

[0192] This example discloses methodology to measure and predict T cell activation in vitro. It was hypothesized that T cell activation in vitro is a function of: B cell and T cell densities (i.e., intracellular distances), B cell target receptor (CD20) expression levels per cell, and bispecific antibody affinities (KD) for target antigens.

[0193] FIG. **6** shows that the T cells are more likely to be activated when B cells had a higher expression level of the antigen CD20.

[0194] Intracellular distances are useful for modeling as T cells that are closer to B cells are more likely to be "activated" in the presence of a bispecific Ab. Intracellular distance was calculated via simulations. The method to calculate distance between B cells and T cells comprised: using R software to simulate experimental cell numbers with random x,y,z coordinates within a cube having a size of 1 μL (1 mm^3); randomly assigning whether a cell was a B or T cell. FIG. **7** shows a simulation of 500 T cells and 500 B cell in 1 μL. For each T Cell (n=500), the average distance (dx) of the 6 closest B Cells was determined. Furthermore, the Overall Average Distance (Dx, in mm) from the previous step was determined to reach a final average distance value. FIG. **8** shows simulations of Intracellular distance between T cells and B cells. FIG. **9** shows that T cells closer to B cells are more likely to be activated.

[0195] In summary, higher B cell target expression levels and shorter intracellular distance between T cells and B cells both lead to enhanced T cell activation.

[0196] In addition to the various embodiments depicted and claimed, the disclosed subject matter is also directed to other embodiments having other combinations of the features disclosed and claimed herein. As such, the particular features presented herein can be combined with each other in other manners within the scope of the disclosed subject matter such that the disclosed subject matter includes any suitable combination of the features disclosed herein. The foregoing description of specific embodiments of the disclosed subject matter has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the disclosed subject matter to those embodiments disclosed.